

The identification and estimation of nitric oxide by its absorption in alkaline sodium sulphite

Alkaline sodium sulphite¹ has been widely used as an absorbent for nitric oxide. This gas has been identified as the product of a nitrite-reductase system derived from *Pseudomonas aeruginosa* through its stoichiometric absorption by this reagent².

The gaseous products of the anaerobic incubation of sodium nitrite with fresh minces of pig muscle at pH 6.0, showed an increase in the absorbancy (in the ultra-violet region below 290 m μ) of the alkaline sulphite absorption reagent after 100-fold dilution. Mass spectrometric and infrared spectrophotometric evidence for the presence of nitric oxide in these gaseous products prompted the treatment of the alkaline sulphite reagent with this gas; again, after 100-fold dilution of the product in order to reduce the background absorption of the reagent to a negligible density, an increase of absorbancy in the ultraviolet region was obtained.

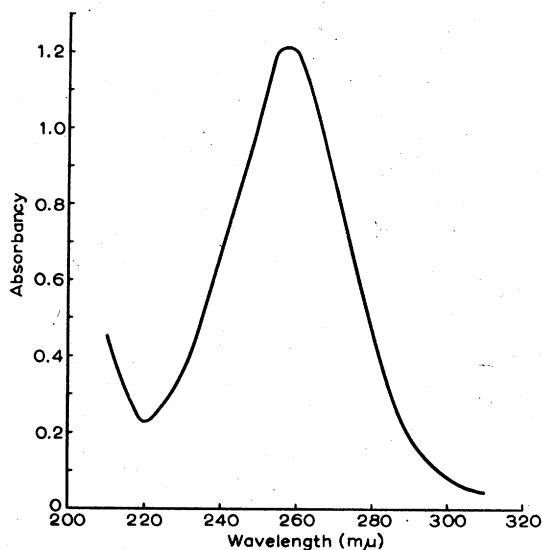


Fig. 1. Absorption spectrum of potassium *N*-nitrosohydroxylamine-*N*-sulphonate freshly dissolved in aqueous potassium hydroxide (0.02 %, w/v). Concentration, 5.0 mg percent; light-path, 1.0 cm.

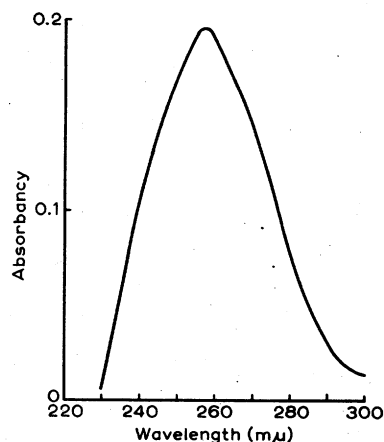


Fig. 2. Absorption spectrum of solution of nitric oxide in aqueous potassium hydroxide (0.5 %, w/v). Light-path, 1.0 cm.

The product of interaction of nitric oxide and alkaline sulphite, potassium *N*-nitrosohydroxylamine-*N*-sulphonate, was prepared according to the method of NYHOLM AND RANNITT³. The white powder obtained decomposed rapidly in aqueous solution with evolution of gas and loss of absorption in the ultraviolet region. The powder was more stable in alkali, the half-life period of its exponential decay in potassium hydroxide solution (0.02 %, w/v) being of the order of 45 min. Its absorption spectrum when freshly dissolved in this solvent is shown in Fig. 1; the peak at 258 m μ was masked in solution in alkaline sulphite, since the absorbancy of the solvent rose rapidly below 260 m μ after 100-fold dilution. Gradients of absorbancy per μ g of nitrogen/ml (Dumas determination by Drs. WEILER AND STRAUSS, Oxford)

of 0.23 at 258 $m\mu$ and 0.17 at 270 $m\mu$ were obtained for fresh solutions of potassium *N*-nitrosohydroxylamine-*N*-sulphonate in 0.02 % potassium hydroxide. The relationship was linear at each wavelength up to a concentration of 2.5 μg of nitrogen/ml; this represents a millimolar extinction coefficient, at the 258- $m\mu$ peak, of 6.5 on the basis of two atoms of nitrogen per molecule. Only 37 % of this nitrogen was, however, recovered as ammonia on reduction with Devarda's alloy in Conway units, probably because of the conversion to hydrazine derivatives which has been reported to occur with reducing agents³.

No change was observed in the absorbancies at 270 $m\mu$ of portions of a solution of nitric oxide in alkaline sodium sulphite successively removed at intervals up to 2.5 h, diluted 100-fold with water and examined immediately. The absorbancies of the diluted portions themselves subsequently decayed in a similar manner to the solutions of potassium *N*-nitrosohydroxylamine-*N*-sulphonate in alkali.

Of the other gases likely to be formed during reduction of nitrate or nitrite, nitrous oxide was found to reduce the absorbancy of alkaline sodium sulphite in the ultraviolet region. Nitrogen dioxide raised the absorbancy of the reagent slightly, the increase at 270 $m\mu$ being 0.0019 per μg of nitrogen/ml in the 100-fold diluted solution according to volumetric estimations of the uptake of the gas in a Haldane gas analysis apparatus; this increment in absorbancy was in agreement with that obtained for mixtures of sodium nitrite and potassium nitrate in equimolecular proportions. The saturation of alkaline sodium sulphite with carbon dioxide did not materially affect its absorbancy in the ultraviolet region after 100-fold dilution with water.

The bubbling of nitric oxide, washed once with concentrated sulphuric acid and twice with alkali, through potassium hydroxide solution (5 %, w/v) resulted in a solution having, after 10-fold dilution with water, elevated absorbancy in the ultraviolet region, again with a maximum at 258 $m\mu$ (Fig. 2). The gradient of the linear relationship obtained between the nitrogen in solution in 0.5 % potassium hydroxide (determined as ammonia after reduction with Devarda's alloy in Conway units and assuming a 67 % recovery of nitrogen as obtained from nitrite and nitrate under similar conditions) and the increase in absorbancy at 258 $m\mu$ was 0.0092 per μg of nitrogen/ml at least up to 10 μg of nitrogen/ml; no reduction was observed in the absorbancy, at 258 $m\mu$, of solutions of nitric oxide in 0.5 % potassium hydroxide over at least 3 h, and no nitrite capable of diazotising sulphanilic acid⁴ could be detected in solution.

In view of the negligible or even contrary effects of other possible gaseous products, increases in absorbancy in the ultraviolet region of the spectrum (particularly at 270 $m\mu$) of alkaline sulphite absorbents after 100-fold dilution can be used to identify and estimate small volumes of nitric oxide arising from the biochemical reduction of nitrite and nitrate. Moreover, manometric estimations of nitric oxide by differential absorption in alkali and in alkaline sodium sulphite are low owing to the solubility of the gas in both absorbents which precludes the absolute differentiation of small volumes of nitric oxide and carbon dioxide. The anaerobic incubation of minces of fresh pig muscle at pH 6.0 with a high concentration (0.43 %, w/v) of sodium nitrite, for instance, yielded an alkali-insoluble, but alkaline sulphite-soluble, gas fraction which was severely reduced (or even eliminated) on dilution of the nitrite concentration 100 fold. Nevertheless, increases in the absorbancies of the alkali and

alkaline sulphite absorbents showed that nitric oxide was produced at the lower nitrite level. Of 170 μg of sodium nitrite incubated anaerobically for 90 min with a mince of muscle, for example, an average of 44 μg (26 %) was recovered unchanged from the incubation medium. Using an adaptation of the method of HORNSEY⁵, the equivalent of 8.4 μg (5.5 %) was estimated to be in combination as nitrosyl myoglobin, and nitric oxide equivalent to 98 μg (58 %) was estimated to be in the alkaline sulphite absorbent.

This work forms part of a research project being carried out with funds made available through the U.S. Department of Agriculture under U.S. Public Law 480. We are grateful to Mrs. C. BROWN, B.Sc., and to Messrs. R. OPIE and P. F. SQUIRES for their skilled technical assistance.

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Received September 9th, 1963

Biochim. Biophys. Acta, 82 (1964) 423-425